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Space Administration

August 4, 2000

NRA-00-HEDS-03

NASA
RESEARCH ANNOUNCEMENT

Human Exploration and Development of Space (HEDS)

Cellular and Macromolecular Biotechnology

Notices of Intent Due: September 6, 2000

Proposals Due: October 27, 2000

OMB Approval No. 2700-0087

CELLULAR AND MACROMOLECULAR BIOTECHNOLOGY

NASA Research Announcement
Soliciting Research Proposals
for the Period Ending
October 27, 2000

NRA-00-HEDS-03
Issued: August 4, 2000

Office of Life and Microgravity Sciences and Applications
Human Exploration and Development of Space (HEDS) Enterprise
National Aeronautics and Space Administration
Washington, DC 20546-0001

NASA RESEARCH ANNOUNCEMENT

Cellular and Macromolecular Biotechnology

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NASA RESEARCH ANNOUNCEMENT

CELLULAR AND MACROMOLECULAR BIOTECHNOLOGY

This NASA Research Announcement (NRA) solicits proposals to conduct scientific investigations in the areas of macromolecular and cellular biotechnology that support the Human Exploration and Development of Space Enterprise (HEDS). These investigations may involve ground-based research or flight experiments intended to support NASA's research goals. Further descriptions of the biotechnology research activities are given in Appendix A.

Investigations selected for flight experiment definition must successfully complete subsequent development steps to be considered for a flight assignment. NASA does not guarantee that any investigation selected for definition will advance to flight experiment status. Investigations selected for support as ground-based research generally must propose again to a future solicitation in order to be selected for a flight opportunity or be reviewed for transition from ground-based research to flight research.

Participation is open to U.S. and foreign investigators and to all categories of organizations: industry, educational institutions, other nonprofit organizations, NASA centers, and other U.S. Government agencies. **Though NASA welcomes proposals from non-U.S. investigators, NASA does not fund principal investigators at non-U.S. institutions** (See Appendix A). Proposals may be submitted at any time during the period ending October 27, 2000. Late proposals will be considered if it is in the best interest of the Government. Proposals will be evaluated by science peer reviews and engineering feasibility reviews. It is planned for selections to be announced by February 2001 and grants or contracts awarded shortly thereafter.

Appendices A and B provide technical and program information applicable only to this NRA. Appendix C contains general guidelines for the preparation of proposals solicited by an NRA.

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This NRA is available electronically at the following web site:

http://peer1.idi.usra.edu/

Notices of Intent should be submitted electronically at the above web site. If electronic means are not available, you may mail Notices of Intent to the address given below.

Proposals are to be submitted to the following address:

Dr. Steve Davison
c/o NASA Peer Review Services
Subject: NASA Research Proposal (NRA-00-HEDS-03)
500 E Street SW, Suite 200
Washington, DC 20024
Telephone for delivery services: (202) 479-9030, Ext. 200

NASA cannot receive proposals on Saturdays, Sundays, or federal holidays.

Proposal copies required: 15

Proposers will receive an e-mail confirming receipt of proposal within 10 working days of the due date.

Obtain Programmatic Information about this NRA from:

Dr. Steve Davison
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NASA Headquarters
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Selecting Official: Director
Microgravity Research Division
Office of Life and Microgravity Sciences and Applications
NASA Headquarters

Your interest and cooperation in participating in this effort are appreciated.

SIGNED AS PER ORIGINAL

Kathie L. Olsen, Ph.D.
Acting Associate Administrator
Life and Microgravity Sciences and Applications

Technical Description

CELLULAR AND MACROMOLECULAR BIOTECHNOLOGY

I. INTRODUCTION

A. BACKGROUND

The Human Exploration and Development of Space (HEDS) Enterprise, one of four National Aeronautics and Space Administration (NASA) strategic enterprises, conducts a program of basic and applied research to improve the understanding of fundamental physical, chemical, and biological processes. The HEDS Strategic Plan defines four major objectives: (1) prepare to conduct human missions of exploration to planetary and other bodies in the solar system; (2) use the environment of space to expand scientific knowledge; (3) provide safe and affordable human access to space, establish a human presence in space, and share the human experience of being in space; (4) enable the commercial development of space and share HEDS knowledge, technologies, and assets that promise to enhance the quality of life on Earth. In support of the HEDS objectives, the current NRA will be used to solicit research in the areas of cellular and macromolecular biotechnology that support development of research for the International Space Station and applied research in biotechnology for long-duration space flight.

The Microgravity Research Division of the Office of Life and Microgravity Sciences and Applications (OLMSA) is an integral element of the Human Exploration and Development of Space (HEDS) Enterprise. The scope of the program, sponsored by the Microgravity Research Division (MRD), ranges from applied research to support HEDS objectives, to technology development, to basic research that uses low gravity to address fundamental research questions. This Biotechnology announcement is being released as part of a coordinated series of discipline-directed solicitations intended to span the range of the MRD program. Other MRD solicitations planned for periodic release encompass the areas of combustion science, fluid physics, fundamental physics, and materials science.

The scope of this research announcement does not include research dealing with the response of living organisms to weightlessness, an area which is the focus of an ongoing program in the Life Sciences Division.

B. RESEARCH ANNOUNCEMENT OBJECTIVES

NASA's Biotechnology Programs support the development of new science and technology research under the HEDS Enterprise. The programs promote U.S. competitiveness and insure NASA leadership in providing cutting edge scientific research and technologies for space missions. The program also seeks a coordinated research effort involving both space- and ground-based research. This NRA has the objective of broadening and enhancing the program through the solicitation of:

- Experimental studies which require the space environment to test clearly posed hypotheses, using existing or slightly modified flight instruments;
- Experiment concepts which will define and use new instruments for space-based experiments;
- Ground-based theoretical and experimental studies which will lead to the definition of potential flight experiments or to enhancement of the understanding of existing investigations; and

- Ground-based studies with emphasis on research leading to technologies required by future human space missions.

Further programmatic objectives of this NRA include objectives broadly emphasized by the civil space program, including: the advancement of economically significant technologies; technology infusion into the private sector; and enhancement of the diversity of participation in the space program, along with several objectives of specific importance to the microgravity science research program. These latter objectives include the support of investigators in early stages of their careers, with the purpose of developing a community of established researchers for the International Space Station and other missions in the next 10-20 years.

In support of the HEDS Enterprise, individuals participating in the MRD Program are encouraged to help foster the development of a scientifically informed and aware public. Therefore, all participants in this NRA are asked to promote general scientific literacy and public understanding of the value of scientific research. Where appropriate, investigators will be required to produce, in collaboration with NASA, a plan for communicating to the public the value and importance of their work.

C. DESCRIPTION OF THE ANNOUNCEMENT

With this NRA, NASA is soliciting proposals to develop research in cellular and macromolecular biotechnology for the International Space Station or conduct applied research for long-duration space flight. The goals of the program along with several research areas of interest are described in Section II.

The program seeks a balanced research effort involving both space- and ground-based research. Ground-based research forms the foundation of this program, providing the necessary experimental and theoretical frameworks for development of rigorous understanding of basic phenomena. This research can eventually mature to the point where it becomes the focus of a well-defined flight experiment or supports development of new technologies for future NASA missions. Ground-based research efforts are generally supported for a period of four years; however, shorter periods of support may be appropriate for proposals that are more exploratory in nature.

NASA is currently developing flight instruments for microgravity biotechnology research. Brief descriptions of the planned capabilities are given in Appendix B. NASA anticipates future flight opportunities for investigations with requirements which can be met by existing apparatus with only minor modifications. Successful proposals for use of the existing apparatus will be funded for definition studies which will produce a Science Requirements Document (SRD). Authorization to proceed into flight development is contingent upon successful peer review of the experiment and SRD by both science and engineering panels. NASA does not guarantee that any experiment selected for definition which plans to use existing hardware will advance to flight experiment status.

Though researchers should not feel limited by the capabilities of existing flight hardware, it must be emphasized that experiments calling for equipment significantly outside these envelopes will involve considerable more expense to NASA, a factor which must be taken into consideration in funding decisions. Selected proposals requiring development of new capabilities will be funded for flight definition studies to define flight experiment parameters and the appropriate flight hardware. The length of the definition phase will be based on the experiment requirements, but will normally range from 6 to 24 months and will culminate in the preparation of an SRD and a science concept review. NASA does not guarantee that any experiment selected for flight definition which requires new instrument development will advance to flight experiment status. Promising proposals which are not mature enough to allow development of a flight concept within two years of definition may be selected for support in the ground-based research program.

Investigations selected into the ground-based program must generally propose again to a future announcement in order to be selected for a flight opportunity or may be independently reviewed for transition to the flight program. Investigations that do not proceed into flight development will normally be

required to submit a proposal for continuation of support at the conclusion of a typical four-year period of funding.

II. BIOTECHNOLOGY RESEARCH

A. INTRODUCTION

NASA's biotechnology program includes both cellular and macromolecular research. Both of these areas contain promising opportunities for significant advancements through low-gravity experiments. In addition, NASA is expanding the biotechnology program to support Human Exploration and Development of Space (HEDS) activities and specific topics of interest to NASA. Therefore, under this announcement, NASA is inviting proposals for research in these areas.

B. BIOTECHNOLOGY GOALS AND DESCRIPTION OF PARTICIPATION

The goals are 1) to advance the scientific understanding of biotechnology processes relevant to NASA, 2) to use low-gravity experiments for insight into the physical behavior of biotechnology processes, 3) to provide the scientific knowledge needed to improve these processes, 4) to contribute to Earth-based systems concerned with biotechnology, and 5) to develop technologies specifically supporting HEDS activities.

Included in this area are those biotechnological processes which are affected by buoyancy driven convection and sedimentation, and that can be gainfully studied using the low-gravity (diffusion controlled transport) environment of space. Gravity's effect on these processes can be virtually eliminated in space; thus allowing space-based experiments, coupled with ground-based experimental and theoretical research, to provide insights into biotechnological processes.

One objective of this announcement is to solicit investigations in biotechnology which will establish the scientific foundations for future flight experiment development. Proposals for flight experiments which are mature and could proceed to flight using simple hardware designed to work inside the glovebox (see Appendix B) or using existing hardware with little modification are also solicited under this announcement.

To accomplish these goals, this research announcement is soliciting three types of proposals for all areas of biotechnology research:

I) Flight proposals to carry out experimental research in the space environment

- Experimental studies which require the space environment to test clearly posed hypotheses, using existing or slightly modified instruments;

- Experiment concepts which will define and utilize new instruments for space-based experiments in biotechnology.

Investigations proposing flight experiments should be sufficiently mature from a scientific and technical standpoint to define a flight investigation and proceed to a review of their concept within two years of their initial funding. Several possible levels of participation are envisioned for flight investigations: (1) proposers may offer to design and develop instruments under contract with NASA, (2) proposers may offer to use existing hardware, (3) proposers may offer to develop simple hardware that can be used within the capabilities of the glovebox, or (4) proposers may offer research to be performed in NASA developed or international instruments with the proposer providing scientific guidance to the development effort (see Appendix B for instrument descriptions).

II) Ground-based research proposals

- Ground-based theoretical and experimental studies which will lead to the definition of potential flight experiments or enhance the understanding of existing investigations.

Research proposals are requested that define and characterize phenomena and processes for which microgravity represents an enabling environment. The program has as its near-term objective the development of a knowledge base sufficient to assess the scientific value of experiments in biotechnology under low gravity conditions.

III) Interdisciplinary research projects that can support development programs and novel research approaches

- This research announcement is also soliciting a third type of proposal for both Macromolecular and Cellular Biotechnology that encourages the formation of teams of researchers where biological researchers and researchers from other areas (optics, materials science, fluid dynamics, bioengineering, etc.) can advance the research through an interdisciplinary approach.

Research groups from the same or from different institutions may team and submit a joint research proposal. Proposals in this category must be formed through a cooperative arrangement between the research groups with one research group having, for example, comprehensive bioengineering and fluid dynamic capabilities and the other an outstanding background in the biological sciences. The goals of the interdisciplinary research projects are to develop advanced technology, promote ground-breaking research, and support technology transfer. Because biotechnology is an inherently cross-disciplinary activity, interdisciplinary proposals are being solicited under this announcement to produce research teams with a critical size to accelerate areas of interest to NASA. These interdisciplinary proposals will allow the teaming of science research groups to address complex problems.

Interdisciplinary proposals should identify key personnel and their expertise. It must be clearly stated who the Principal Investigator and the lead institution are and how the effort will be integrated (see Appendix C). A science team, for example, may wish to work with a strong engineering team, at its own or another institution, but these proposals should state how teaming and cooperation between the engineering and science teams will be managed.

Management structure, goals, and cooperation with the research community to facilitate the transfer of technology must be evident in the proposal.

C. CELLULAR BIOTECHNOLOGY

NASA's Cellular Biotechnology Program is developing new technologies for the maintenance and growth of three-dimensional mammalian tissues. The program addresses the development of rotating vessel bioreactors for the culture of cells using well-controlled process parameters and reduced levels of hydrodynamic stress, thus providing a model system for the low gravity conditions of space to the extent possible on Earth. Under this solicitation, NASA is seeking to expand its activities in bioreactor design and tissue engineering with the goal of developing tissue models for space experiments and biomedical research.

Research demonstrates that the stresses to which cells are subjected should be minimized to culture cells with a low rate of morbidity and promote efficient assembly of cells into tissue constructs. NASA's rotating bioreactor uses cell suspension to yield shear stresses for cell aggregates that are significantly smaller than those in conventional, stirred bioreactors. Mammalian cells cultured in this low shear environment aggregate and grow into relatively large masses, and the cultured cells display differentiation markers similar to those found in *in vivo* mammalian tissues. NASA's basic research component of this program is to understand the role of reduced hydrodynamic stress and microgravity on mammalian cell adhesion, proliferation, and eventual differentiation. In space, the mass transport to and from cells and the shear stresses on cultured cells is greatly affected by the lack of sedimentation in a microgravity environment.

The program has the following major objectives concerning mammalian tissue culture: 1) accelerate the development of advanced three-dimensional tissue culturing systems, 2) define and characterize mammalian cells and tissues that benefit from a low shear environment, 3) use the microgravity environment of space as necessary to surmount the obstacles to the propagation of complex tissues, and 4) Develop tissue models that support biomedical research in space and on Earth. Flight research involves tissue systems that demonstrate enhanced growth and differentiation in ground-based rotating vessel research. NASA is carrying out preliminary development work on flight hardware capable of supporting short and long duration mammalian tissue culture studies (see Appendix B for instrument descriptions). The following research areas are central to the Cellular Biotechnology program:

- TISSUE ENGINEERING

Millions of Americans suffer tissue or organ loss from diseases and accidents every year, and the yearly cost of treating these patients exceeds $400 billion. The major medical treatment for these losses is transplantation of tissues and organs; however, these transplantations are severely limited by donor shortages (R. Langer and J. Vacanti, *Science* 260, 920, 1993). The shortages of replacement tissue and organs have generated a substantial research effort on the development of alternative sources for transplantations. Improved cultivation of cells and tissues so that the processes of organ failure and organogenesis are better understood may yield better approaches to either avoid pathological organ failure or allow the creation of new replacement tissues and organs for transplantations.

Ground-based research studies demonstrate that both normal and neoplastic cells and tissues recreate many of the characteristics in the NASA bioreactor that they display *in vivo*... Proximal renal tubule cells that normally have rich apically oriented microvilli with intercellular clefts in the kidney do not form any of these structures in two-dimensional monolayer culture. However, when normal proximal renal tubule cells are cultured in three-dimensions in the bioreactor, both the microvilli and the intercellular clefts form. Similar results in recreating normal structures have occurred in cartilage cultures using the NASA bioreactor technology.

The microgravity environment affords a unique opportunity to culture cells because they may be grown without sedimentation. This provides the setting to recreate the three-dimensional relationships among cells that are extremely important to normal tissue morphogenesis and thereafter, organ function. Freed *et al*. (*Proc. Natl. Acad. Sci*... 94, 13885-13890, 1997) reported results from the first long-duration tissue engineering experiment conducted in the microgravity environment of space. The experiments validated tissue morphogenesis in space and demonstrated unique characteristics of tissue produced in the microgravity environment of space.

- TISSUE MODELS FOR HUMAN DISEASE STUDIES AND DRUG TESTING

In studies with malignant cells, investigators recreated the morphologic appearance of tumor-like structures that secrete products related to metastasis to liver by human colon carcinoma, to bone by human prostate carcinoma cells, or to various organs by other carcinomas. These studies generally involve co-cultivation of the malignant cell with the normal cells that form the matrix and provide signals necessary for the three-dimensional shape of the cancerous tissue and that it promotes the production of factors that are not detectable in conventional bioreactor cultures.

The importance of three-dimensional culture is evident because when the appropriate tissue morphology is recreated, function is more likely to follow. Studies have showed that three-dimensional cultures of many different types of malignant cells are resistant to chemotherapy that normally kills these tumor cells in two-dimensional monolayer culture. Thus, the ground-based studies with malignant cells that recreate the morphological appearance and behavior of metastases in an animal or man may provide a superior test format for the development of new therapeutic agents as well as improving our understanding of how prior chemotherapy might be made more effective.

The NASA/NIH Center for Three-Dimensional Tissue Culture (located at the National Institute of Child Health and Human Development) supports the transfer of NASA bioreactor technology to the NIH and its application to biomedical research. The center is pursuing fundamental investigations that are of medical and biological importance. Researchers have used the bioreactor technology in tissue engineering and a wide range of infectious disease studies; significant results include the following:

- The first three-dimensional lymphoid tissue system permitting the study of the human immunodeficiency virus (HIV-1) pathogenesis through human monocytes and lymphocytes. To understand HIV pathogenesis, cultures of functionally active human lymphoid tissue were established using the NASA Bioreactor. Scientists use tonsil tissue to grow live Human Immunodeficiency Virus (HIV-1) and thus observe more closely the transmission of the virus. The main events in HIV pathogenesis occur inside lymphoid tissue, and the cultures demonstrated the same progressive loss of CD4 T-cells as seen in AIDS patients. This tissue system may also provide the potential for rapid screening of therapeutics and identifying key stages in HIV pathogenesis to target for vaccine or chemotherapy.

- The center also developed culture methods for infectious pathogens using the NASA bioreactor technology. These research groups have already shown that the bioreactor can support the culture of Cyclospora, intestinal parasites found on fruit. The bioreactor technology enabled its culture and the possibility of studying this parasite. In addition the center has successfully cultured the Borrelia burgdorferi, the parasite responsible for Lyme disease.

Research in this area will help establish the scientific basis for conducting culture experiments in the microgravity environment of space, contribute to the culture of functional and differentiated tissues for use in medical treatments, and will contribute to advances in developmental biology.

- BIOREACTOR DESIGN

The physical environment that cells and tissue are cultured in has a dramatic effect on the differentiation of the cells and the resulting tissue architecture. NASA is coupling fluid physics, biomaterials, and automated control systems into its tissue engineering program with the goal of generating novel bioreactor designs with enhanced transport and appropriate fluid shear properties to support the growth of complex tissue constructs. Research topics included in this area are as follows:

- Bioreactor technologies for tissue engineering.

- Research into new tissue culture technologies and approaches that support three-dimensional tissue growth. Perfusion methods, pulsed-flow approaches for blood vessels.

- Research on the effect of reduced levels of mechanical and hydrodynamic shear, spatial co-location of participating cell populations, and the role of mass transport on cellular propagation and tissue assembly in rotating wall bioreactor systems.

- Research that offers new tissue culturing methods and strategies that produce three-dimensional tissue propagation.

- STUDIES ON CHANGES IN GENE EXPRESSION AND METABOLIC FUNCTION

Gene array analysis of cultured human renal cells was used to do the first comprehensive analysis of the effects of the microgravity environment of space on the expression of 10,000 genes (T. Hammond et al., Nature Medicine Volume 5, Number 4, 1999). In this case, more than 1600 genes in the kidney cells changed their expression levels up and down in microgravity, demonstrating that there is a select group of gravity dependent genes.

Renal cells were grown in microgravity, on the ground using the rotating wall vessel culture system, and in a centrifuge at 3 G. While only 5 genes changed more than 300% during 3g centrifugation, 1,632 genes

changed in microgravity, and 914 genes changed in the rotating wall vessel. Thus, renal cells display discrete responses to changes in their gravitational environment during growth.

Even more importantly, this research demonstrates the use of space as a potential probe to discover new cellular regulatory systems and reactions. Such discoveries can open new strategies in understanding disease processes and advance our knowledge of cellular processes. Thus, the unique environment of space is a logical extension of our laboratories on Earth and holds opportunities for novel discoveries about human cells.

- OTHER RESEARCH AREAS OF INTEREST TO NASA'S CELLULAR BIOTECHNOLOGY PROGRAM

    - Investigate the physical environment produced in the bioreactor as a model for understanding the cellular response to microgravity. This research may lead to a more expeditious use of the microgravity environment in tissue engineering and cell culture.

    - Assess the value of low shear and spatial co-location culturing by establishing functional tissue to do morphological analysis using rotating wall vessels. This research must be able to quantitatively measure determinable parameters such as tissue mass, tissue differentiation or diversification markers, tissue function, or production of biologically active materials.

    - Research the effects of culture media (growth factors, etc.), cellular metabolism, and waste accumulation to facilitate the propagation of differentiated, functioning tissues in space and ground-based bioreactors.

    - Biomaterials and scaffolds for tissue engineering: biopolymer and biomaterials research that support growth of anchorage dependent cells and allows tissue to from with the correct morphology.

    - Research to support the development of technologies (biosensors for pH, glucose, and oxygen levels, etc.) and maintenance strategies for three-dimensional tissue culture to allow long-term automation and improve the tissue culturing process via a more physiologically balanced system.

D. MACROMOLECULAR BIOTECHNOLOGY

NASA's macromolecular biotechnology research program encompasses a spectrum of research including structural biology studies of biological molecules and assemblies, biomaterials research, biological nanotechnology, and other areas of macromolecular research which can be better understood using the microgravity environment of space or have application to the HEDS research objectives (see section I). The program has the following goals: 1) extend crystallographic analyses to more complex and challenging systems, such as motor proteins, glycoproteins, and integral membrane proteins by using the space environment, 2) elucidate the fundamental factors that provide for the observed benefits in diffraction performance when macromolecular crystals are grown in microgravity, 3) develop technologies and quantitative methodologies that will improve structural biology research, 4) support research and development of leading-edge technologies for NASA missions in biomaterials, biological nanotechnology, and self-assembling materials .

- STRUCTURAL BIOLOGY RESEARCH

<u>Research on Challenging Structural Biology Problems</u>

A recent NRC report (see bibliography) has recommended that NASA fund a series of high-profile grants to support research that uses microgravity to produce crystals of macromolecular assemblies with important implications for cutting-edge biology problems.. The NRC report stated that the success or

failure of these research efforts would definitively resolve the issue of whether the microgravity environment can be a valuable tool for structural biology.

Therefore, request for structural biology research proposals that address the NRC report recommendations are a program priority. Some examples of classes of systems that currently meet these criteria include the following: membrane proteins, molecular motors, biopolymer synthetic machinery (e.g., origin of replication complexes and transcriptional pre-initiation complexes). The NRC report described all of these systems as elaborate and fragile, which makes them difficult to crystallize unless the conditions are just right; microgravity might improve the quality of the crystals enough to lower the resolution to a level at which key structures can be discerned. For example, macromolecular assemblies that have been crystallized to 4 angstroms could benefit if microgravity crystallization allowed improvement to 3.5 angstroms where alpha helices and beta sheets could be discerned.

Under this area, macromolecular systems where efforts are already underway, but crystallization has been difficult and the results have been borderline, would be favored over nascent research projects.

Neutron Diffraction Studies

Advances in neutron diffraction in combination with the availability of new neutron sources have lead to the possibility of collecting improved neutron diffraction data on biological macromolecules. Structural determination by neutron diffraction requires much larger, high quality crystals on the order of millimeters. Spaceflight experiments have frequently returned diffraction quality crystals of large sizes, and often larger than those obtained on the ground. The program hopes to obtain higher resolution of structures or the solution of structures of more complex materials through the use of crystal growth in a microgravity environment.

- STRUCTURAL BIOLOGY CRYSTALLIZATION STUDIES AND TECHNOLOGIES

Crystal growth in a microgravity environment can have beneficial effects on the size and intrinsic order of macromolecular crystals. In many cases, crystals obtained in space are larger, have lower mosaicity, and diffract to higher resolution than comparable crystals grown on the Earth. In order to build on these results, NASA is supporting research to study the crystallization of biological molecules and assemblages.

Research in this area should be largely directed at supporting the biologically challenging problems mentioned above (membrane proteins, molecular motors, biopolymer synthetic machinery, etc.), or the scope of the proposed research broad enough to support generalizations to these types of systems. This research program will provide a framework for understanding microgravity crystallization results, optimizing growth conditions for biological crystals, characterizing defect formation and its relationship to growth mechanisms, and provide a more rational approach to the growth of macromolecular crystals.

Research may involve atomic force microscopy, laser light scattering, interferometry, and x-ray topography to better relate growth conditions and defect formation, and to correlate observations with various measures of crystal quality such as mosaicity and diffraction resolution. This research may allow the quantification of essential kinetic parameters, delineation of relevant mechanisms, and the identification of optimal macromolecular samples for space experiments.

To increase the impact of this program on structural biology, NASA is also inviting proposals that develop technologies to improve macromolecular crystallization throughput for ISS structural biology research and proteomics research. Technologies for monitoring, controlling, and automating crystal growth of biological macromolecules are core technologies that can accelerate progress in structural biology. These types of technologies could be used to facilitate the growth of macromolecular crystals in space or enhance throughput for large-scale screening and data collection on the ground. Therefore, robotic and automated crystal growth technologies, screening methods, and cryopreservation techniques that can support high throughput crystallization are requested under this research announcement.

Coupled to the ability to do high throughput crystallization is the rational prediction of crystallization conditions for a given macromolecule and the optimization of these conditions. Predictive and optimization technologies that allow the best set of conditions to be rapidly determined are important for progress in structural genomics. Optimization technologies may also include the development and use of sophisticated databases for optimizing or predicting crystal growth conditions.

- BIOLOGICAL NANOTECHNOLOGY

Biological nanotechnology can be used to reduce NASA mission requirements such as weight, volume, electrical power, etc. Biological molecules can be incorporated into nanotechnology development in several ways: biological molecules or assemblies could serve as molecular-sized sensors, genetic engineering could be used to produce a single molecule with both a molecular receptor and a signal function, etc. In addition, these systems may be able to be produced very reliably and economically because biological systems are capable of manufacturing large molecules in a highly reproducible manner, far more reproducible than any corresponding industrial processes.

One example of the unique types of sensors that can be developed is demonstrated by the light sensitive molecules bacteriorhodopsin and chlorophyll. These molecules are among the most efficient photon receptors and information transmitters known. For example, the eye detects and reports single photon reception. In principle, biologically produced compounds of this nature can be used for detection of light, circuitry, switching, and memory applications. Genetically engineered cells could be used to tailor naturally produced compounds for specific applications.

Biological nanotechnologies may also be able to take advantage of the built in signaling logic associated with many types of subcomponents of cells. These macromolecules and assemblies often are able to react to multiple chemical, electrical, and light stimuli. As a result they are capable of behavior more complex than a standard digital device. Harnessing these biological systems and use of their capabilities would accelerate the development of artificial intelligence.

Engineered protein crystals offer the possibility of new classes of materials, ordered on nanometer scales, that can be exploited initially for practical applications such as ultrafiltration membranes and solid enzyme catalysts. This research is also a first step in learning how to fabricate complex, self-assembling protein devices that lead to even more complex bio-based "nano-devices" such as tiny motors, sensors, and energy transducers.

Other areas of interest include technologies to manipulate biological molecules to form useful devices or nano-scale structures. The applications of this new technology are to areas such as membrane rheology, micro-scale particle sorting, and two-dimensional macromolecular array formation of proteins and DNAs.

- BIOMOLECULAR SELF-ASSEMBLING MATERIALS

Research on biomolecular self-assembling materials lies at the interface between molecular biology, the physical sciences, and materials engineering. A key feature of biomolecular materials, such as biological membranes, is their ability to undergo a self-assembly process forming a hierarchical structure without external intervention. Understanding nature's principles and mechanisms used in forming self-assembled structures can lead to their application in the development of new processes and formation of unique materials with significant technological impact. Examples of research in the areas of biomolecular self-assembling materials include: polymer biosynthesis, self-assembled monolayers and multilayers, decorated membranes, mesoscopic organized structures, and biomineralization. Biomolecular self-assembling materials may provide novel properties and applications in life support or other areas central to HEDS goals.

Included in this area is two-dimensional array formation that can be used as a template for biomaterials applications. These lattices are also of interest due to the unique length scale that biological macromolecules provide, a periodicity on the order of nanometers. Two-dimensional array formation also

has application in the determination of molecular structures by electron diffraction and may prove particularly applicable to membrane proteins, molecules that naturally form two-dimensional structures.

- BIOMATERIALS: STRUCTURAL PROTEINS

Structural proteins such as collagen, keratin, and silk may form the basis for producing a variety of hierarchical structures that are characterized by the ability to undergo biomolecular self-assembling. Structural protein-based materials may be able to incorporate novel properties by genetically engineering the sequences or incorporating modular components from other proteins. In addition, since these materials could be produced using recombinant DNA technology, a uniform and controllable architecture is possible. NASA is currently supporting research on genetically engineered elastomeric proteins, natural protein biomaterials that display elastic behavior in their normal biological function. This research is designing synthetic protein polymers based on the elastomeric repeat sequence of this natural elastin.

Under this research announcement, NASA is looking to expand its current activities in protein-based materials. This research may focus on the production of a protein-based material or its isolation from cells in a useable form. These biomaterials may have application to the HEDS research objectives and future NASA missions.

E. PHYSICAL SCIENCES RESEARCH RELATED TO BIOTECHNOLOGY

Expansion of biological technologies requires an understanding of the fundamental processes on which these technologies are based. NASA is currently defining a program that will study those processes which are affected by buoyancy driven convection and sedimentation, and that can be gainfully studied using the low-gravity (diffusion controlled transport) environment of space. Gravity's effect on these processes can be virtually eliminated in space; thus allowing space-based experiments, coupled with ground-based experimental and theoretical research, to provide insights into biotechnological processes. NASA's goal is to exploit the unique microgravity environment of space to advance the understanding of basic phenomenon, and use the information gained through space experimentation on a wide range of biotechnology applications.

Potential research areas include the following:

1) molecular aggregation, 2) diffusion studies on macromolecules, 3) separation and purification studies, 4) the behavior of electrically-driven flows as related to biological separations, 5) capillary and surface phenomena as applied uniquely to biological systems, and 6) membrane transport phenomena affected by diffusion controlled conditions in microgravity.

Studies on the effects of the acceleration environment experienced in space flight on macromolecular and cellular biotechnology experiments are also invited under this area. NASA is also interested in new technologies that enable the determination of macromolecular physical properties that can benefit both ground-based and flight research. This would include new technologies that enable rapid measurement of relevant physical properties such as solubility, diffusion coefficients, second virial coefficients, etc. are requested under this announcement. This is especially important because few of these properties have been measured for systems associated with biological materials. The rapid measurement of thermophysical properties coupled with numerical modeling produces an integrated approach that would enable the development of improved models.

Proposals for theoretical research in this area connected to, or have an enabling role for investigations which seek to ultimately use the space environment, will also be considered for support through this announcement.

F. BIOTECHNOLOGY RESEARCH IN SUPPORT OF HEDS

The HEDS Enterprise has as a major goal contributing significantly to the opening of the space frontier and expanding the human experience. The focus of the microgravity research program in the HEDS Strategic Enterprise is to foster fundamental understanding of physical, chemical, and biological processes, building a foundation of knowledge that can be applied to Earth- and space-based technologies. Specifically, understanding of the fundamental role of gravity in the space environment in these processes is needed to achieve breakthroughs in science, and to develop enabling technology for exploration of space. The need for improved understanding of biotechnology phenomena and development of new technologies to enable future space technologies and operations should be recognized as one of the opportunities of the discipline.

While basic research is still of major importance to our program, there is shift of emphasis toward mission-oriented research, that is, research aimed at specific problems in biotechnology for space exploration as well as for Earth-based applications.. Thus, it is important that firmer links be developed between the research in support of the exploration of space and practical applications on Earth. In the future, more weight will be placed on relevance to HEDS goals, with linkage of the proposed research to attainment of these goals receiving increased emphasis.

Future proposals are not limited to the topic areas discussed in this Appendix; extension to biotechnology topics not currently included in NASA ‘s Biotechnology Program are strongly encouraged to permit us to broaden the program scope.

Research areas that have been identified as having potential to contribute to the HEDS goals include the following:

- SEPARATION, PURIFICATION, AND REMEDIATION METHODS

Separation and purification methods to clean and recycle water are technologies needed to reduce the costs of exploration in long-duration space flight research. For example, cell and tissue systems require significant amounts of pure water for long-duration space studies. Purification methods must be specific for toxic molecules, reliable, inexpensive, and make small demands on spacecraft resources such as power, mass, and volume. Phase Partitioning and Emulsions: A technique for separation and purification involves emulsions to separate pollutants from an aqueous phase followed by a separation of the

emulsion phase from the aqueous. The emulsion phase often contains chemical constituents which are designed to bind or react with impurities and contain them within the phase of the emulsifier. Preliminary work supported by the Biotechnology discipline has involved flight experiments on both phase partitioning and emulsion studies to determine the relative advantages and disadvantages of performing such chemical processes in orbit..

Remediation of fluids is an important enabling technology for spaceflight. One possible biological application is the use of cellular organisms or biological molecules to convert or catalyze fluid waste to usable products, such as potable water, oxygen, methane, etc. Another application is the ability of biological molecules to bind to specific compounds. This capability can be modified by genetic engineering. Chelating of metals, binding carcinogens, etc. are possible applications. Understanding how variable gravitational levels would affect the efficiency of such systems is critical to developing their potential.

- HEDS CELLULAR BIOTECHNOLOGY

Cells are the basic units of living systems. Many microbial and mammalian cell systems are well characterized in Earth's gravity and we understand some of their responses to environmental changes. These cell systems can afford a basis for analysis of the impact of non-terrestrial environments on basic life forms. The Cellular Biotechnology Program can provide the cell systems and the technology to operate such life-based systems that relay findings from remote unmanned missions or operate in an alert/warning mode for manned spacecraft.

Selective Pressures on Cell Populations
Critical for humanity's long-term occupation of space is the assessment of selective pressures on mammalian and microbial cell populations. The induced phenotypic and genotypic changes need to be assessed through generations of cell populations sustained in the space environment (low gravity, etc.). The development of unique technologies and methodologies to enhance research on selective pressures will apprise us of the risks to our biological integrity and to our life-based support systems.

Development of Biosentinel Technologies
Genome damage from radiation is one primary source of DNA mutation. NASA is seeking new technologies to monitor mutation levels in DNA. Biosensor and biosentinel systems to monitor radiation effects and other space environmental factors (magnetic fields, atmospheres, gravitational fields, etc.) on cell replication and DNA for manned and unmanned robotic science missions are needed. These biosentinel systems for exploratory research may incorporate biomolecules in their detection systems. DNA biosentinel technology will be important for future exploration of space by humans.

MicroBiosensor Monitoring Devices
Micro- and Nano- technology based sensors for use in biological systems and experiments that may have unique application to long-duration space missions. The development of extremely stable and small biosensor devices is central to advancement of many biotechnological processes and their use in support of long-duration space missions.

- APPLICATION OF EXTREMOPHILIC ENZYMES

Certain bacteria and archeabacteria have been found to flourish in extreme environments: high and low temperatures, high and low pH, etc. The unique and robust enzymes produced by these organisms, or the organisms themselves, can have applications in industry and may be of substantial benefit for certain chemical processes involved in long duration space flight or colonization of the Moon and Mars. The specificity of enzymes is superior to conventional catalysts and can serve to eliminate multistep reactions and unwanted side products, making the overall chemical process much more efficient. Research may establish unique application of extremophiles or their enzymes in support of HEDS goals.

- IN SITU RESOURCE UTILIZATION EMPLOYING BIOLOGICAL TECHNOLOGIES

The phrase, in situ resource utilization, has been used to describe the utilization of ores or soils of foreign planets, moons, or asteroids for the production of materials needed for space exploration. Identification or engineering of cells or molecules with the potential to catalyze the production of materials such as oxygen, hydrogen, methane, etc. using alien materials is of interest. Biologically based catalysts are of particular interest since they can be efficient and effective at moderate and low temperatures that are often impractical for standard industrial processes. Biological molecules can be genetically engineered and expressed for the purposes of isolating particular compounds or metals from soil.

- GENETIC ENGINEERING OF BIOMOLECULES FOR LONG-TERM SPACE FLIGHT

Cells and subcomponents of cells are efficient chemical plants reproducibly manufacturing numerous, large, and multifunctional compounds. Genetic engineering allows the manipulation of cells to produce these compounds of specific interest. In most cases, this production is difficult or not possible by standard organic chemistry. Cells such as *Escherichia coli*, yeast, etc. can be engineered to produce one or more compounds. Possible uses for genetically engineered proteins would be nutrients and medicines of value for spacefarers, or a protein-based artificial blood for emergency situations. Ideally, genetically engineered cells would be stored in an inactive state. When needed, the cells could be cultured and the required material expressed.

G. SPECIAL TOPICS IN BIOTECHNOLOGY RESEARCH

- BIOMEDICAL APPLICATIONS OF NASA RESEARCH

NASA research and technology supported under the Microgravity Research Division may have unique application in certain areas of biomedical research that would be of substantial benefit and accelerate a particular area. Under this solicitation, NASA is looking to support investigations that may be collaborative with other agencies in which NASA research or technology is being applied to a particular biomedical research problem. For example, NASA developed laser light scattering technology has found application in biomedical research on eye diseases. One potential area is diabetes-related research that applies NASA technology to address the following areas: a) proliferation of human pancreatic tissues, b) non-invasive glucose sensors, c) encapsulation technologies that support transplantation approaches, d) optical technologies that allow advanced monitoring of eye diseases.

- METABOLIC ENGINEERING

Metabolic Engineering (ME) is the targeted and purposeful alteration of metabolic pathways found in an organism to better understand and use cellular pathways for chemical transformation, energy transduction, and supramolecular assembly. ME typically involves the redirection of cellular activities by the rearrangement of the enzymatic, transport, and regulatory functions of the cell through the use of recombinant DNA and other techniques. ME has many potential applications for NASA long-duration missions where production increased efficiency of complex biomolecules is required. Proposals are invited that develop enabling technologies or potentially useful strategies for employing ME on future NASA missions.

III. EXPERIMENTAL APPARATUS AND FLIGHT OPPORTUNITIES

A. EXPERIMENTAL APPARATUS

In order to address aspects of the research described in Section II, a number of pieces of flight hardware are being developed by NASA. In addition, Appendix B lists the ground-based facilities that are available to support definition studies. NASA also contemplates the development of new research capabilities for biotechnology experiments.

B. FLIGHT OPPORTUNITIES

Limited early flight opportunities under this NRA are expected to include the Space Shuttle and the International Space Station. For the Shuttle opportunities, the experimental hardware will be located in the middeck, allowing astronaut interaction, or in the cargo bay, which does not permit such interaction. Residual acceleration levels on the order of 10^{-4} g are available in the Shuttle for limited periods of time. The Space Acceleration Measurement System is expected to be available to measure and record accelerations near the apparatus for both Shuttle and ISS experiments. Flight durations range from 7 to 16 days for the Space Shuttle. A high-capacity communications network supports Shuttle and payload operations. Downlink channels enable users to monitor their instrument status and science data streams in real time. An uplink channel enables them to act on that information. Experimental apparatus for the early utilization of the International Space Station will be primarily in facilities such as the Glovebox and Express Rack (ISS versions of Shuttle middeck class experiments).

C. EXPERIMENT DEFINITION AND FLIGHT ASSIGNMENT PROCESS

Ground-based research is usually necessary to clearly define flight experiment objectives. Successful proposals for flight opportunities will be supported for a ground-based definition phase before review for flight assignment. The amount of support (technical, scientific, and budgetary) and the length of the definition period (usually from 6 months to 2 years) will depend on the specific investigator needs and the availability of resources from NASA. However, in preparing their budget plan for this research announcement, all respondents should estimate their annual costs for four years.

1. Near-Term Flight Opportunities.

Successful proposals for use of existing instruments will be funded for a period of advanced definition work, after which time the investigator will generate a detailed Science Requirements Document (SRD). The SRD, a detailed experiment description outlining the specific experiment parameters and conditions, as well as the background and justification for flight, must be prepared jointly by a NASA-appointed Project Scientist and the Principal Investigator and submitted for peer review. This formal review by both science and engineering panels will determine if space flight is required to meet the science objectives and if instrument capabilities can be provided to meet the science requirements. Following approval by the review panels, subject to program resources, continuation support will be awarded and the hardware will be modified or developed to meet the science requirements. NASA does not guarantee that any experiment selected for definition will advance to flight experiment status. Investigations with unresolved science or engineering issues at the review of the SRD may be placed in ground-based status with support of limited duration, and the investigator will be asked to submit a proposal to a subsequent solicitation for further support.

2. Future Flight Opportunities.

Successful proposals for the development of new apparatus will be funded for a period of definition. The length of the definition period will be based on the experiment requirements, but will generally be from 6 to 24 months. At the end of the experiment definition phase, the investigator will generate a detailed SRD. Following successful peer review of the SRD by science and engineering panels, the experiment will proceed into flight development and be considered for flight. As with opportunities for existing instruments, NASA does not guarantee that any experiment selected for definition will advance to flight development status, and the possibility exists that investigations may be placed in ground-based status, with continuing support from NASA for a limited period.

3. Ground-Based Definition Opportunities.

Promising proposals for experimental research may be selected for support in the ground-based program. These studies are funded for periods of up to four years. An investigation in the ground-based program must generally propose again to a future announcement in order to be selected for a flight opportunity or the investigation may be independently reviewed for transition to the flight program.

IV. PROPOSAL SUBMISSION INFORMATION

This section gives the requirements for submission of proposals in response to this announcement. The research proposal submitted under this announcement consists of a Principal Investigator who is responsible for all research activities and one or more Co-Investigators. Proposers must address all the relevant selection criteria in their proposal as described in Section VII and must format their proposal as described in this section. Additional general information for submission of proposals in response to NASA Research Announcements may be found in Appendix C.

A. NOTICE OF INTENT (NOI)

Organizations planning to submit a proposal in response to this NRA should notify NASA of their intent to propose by sending a Notice of Intent. The NOI can be submitted via the Internet by clicking on the link to NRA 00-HEDS-03 at the following web site: http://peer1.idi.usra.edu/.

If electronic means are not available, you may mail Notices of Intent to the address given for proposal submission in the following section.

The Notice of Intent, which should not exceed two pages in length, must be typewritten in English and must include the following information:

- The potential Principal Investigator (PI), position, organization, address, telephone, fax, and e-mail address.
- A list of potential Co-Investigators (Co-Is), positions, and organizations.
- General scientific/technical objectives of the research.
- The equipment of interest listed in this NRA, if appropriate.

The Notice of Intent should be received at NASA Headquarters no later than September 6, 2000. The Notice of Intent is being requested for informational and planning purposes only, and is not binding on the signatories. The Notice of Intent allows NASA to better match expertise in the composition of peer review panels with the response from this solicitation. Investigators may also request more detail on the capabilities of the specific equipment that might be utilized to accomplish their scientific objectives.

B. PROPOSAL

The proposal should not exceed 20 pages in length, exclusive of appendices and supplementary material, and should be typed on 8-1/2 x 11 inch paper with a 10- or 12-point font. Extensive appendices and ring-bound proposals are discouraged. Reprints and preprints of relevant work will be forwarded to the reviewers if submitted as attachments to the proposal. In preparation of proposals, the standard forms and certifications should be used. Proposers should prepare cost estimates by year for a period not greater than four years in response to this research announcement.

Fifteen copies of the proposal must be received by October 27, 2000, 4:30 PM EDT to assure full consideration. Treatment of late proposals is described in Appendix C. Send proposals to the following address:

Dr. Steve Davison
c/o NASA Peer Review Services
Subject: NASA Research Proposal (NRA-00-HEDS-03)
500 E Street SW, Suite 200
Washington, DC 20024
Telephone for delivery services: (202) 479-9030, Ext. 200

NASA cannot receive proposals on Saturdays, Sundays, or federal holidays.

Ground research proposals submitted in response to this Announcement must be typewritten in English and contain at least the following elements (in addition to the required information given in Appendix C) in the format shown below, in the following order:

- Form A (Solicited Proposal Application)
- Form B (Proposal Executive Summary). The executive summary should succinctly convey, what it is the proposer wants to do, how the proposer plans to do it, why it is important, how it meets the requirements for microgravity relevance or supports HEDS research objectives. In addition, check the box that defines the proposal as Ground-based, Flight Definition or Interdisciplinary Project.
- Form C (Budget For Entire Project Period Direct Costs Only)
- Form D (Summary Proposal Budget - 1 copy for each year)
- Table of Contents
- Prior Period of Support: **For follow-on proposals of ongoing projects, a summary of the objective and accomplishments of the prior period of support, including citations to published papers, must be included as part of the proposers justification for continued support.**
- Research Project Description containing the following elements:
    - Statement of the hypothesis, objective, and value of this research
    - Review of relevant research
    - A description of the proposed research
    - Microgravity or HEDS research Justification
    - For flight definition proposals, a description of the scientific testing program that is required to determine the flight experiment requirements, and a description of the measurement and analysis techniques planned for use in space.
    - A description of the proposed outreach and education activities
    - Management plan appropriate for the scope and size of the proposed project, describing the roles and responsibilities of the participants.

- Appendices:
    - Supplementary budget information and budget explanations. The cost detail desired is explained below
    - Summary of current and pending support for the Principal Investigator and Co-Investigators from all other funding agencies.
    - Complete current curriculum vita for the Principal and Co-Investigators, listing education, publications, and other relevant information necessary to assess the experience and capabilities of the senior participants.
    - **3.5 inch computer diskette containing electronic copy of Principal Investigator's name, address, complete project title, and executive summary**

Proposal Cost Detail Desired.. Sufficient proposal cost detail and supporting information will facilitate a speedy evaluation and award. Dollar amounts proposed with no explanation (e.g., Equipment: $58,000, or Labor: $10,000) may cause delays in evaluation or award. The proposed costing information should be sufficiently detailed to allow the Government to identify cost elements for evaluation purposes. Generally, the Government will evaluate cost as to if it is reasonable, allowable, and appropriate. Enclose explanatory information, as needed. Each category should be explained. Offerors should exercise prudent judgment as the amount of detail necessary varies with the complexity of the proposal.

V. GUIDELINES FOR INTERNATIONAL PARTICIPATION

(1) NASA welcomes proposals from outside the U.S. However, foreign entities are generally not eligible for funding from NASA. Therefore, unless otherwise noted, proposals from foreign entities should not include a cost plan unless the proposal involves collaboration with a U.S. institution, in which case a cost plan for only the participation of the U.S. entity must be included. Proposals from foreign entities and proposals from U.S. entities that include foreign participation must be endorsed by the respective government agency or funding/sponsoring institution in the country from which the foreign entity is proposing. Such endorsement should indicate that the proposal merits careful consideration by NASA, and if the proposal is selected, sufficient funds will be made available to undertake the activity as proposed.

(2) All foreign proposals must be typewritten in English and comply with all other submission requirements stated in the NRA. All foreign proposals will undergo the same evaluation and selection process as those originating in the U.S. All proposals must be received before the established closing date. Those received after the closing date will be treated in accordance paragraph (g) of this provision. Foreign sponsors may, in exceptional situations, forward a proposal without endorsement if the endorsement is not possible before the announced closing date.. In such cases, the NASA sponsoring office should be advised when a decision on endorsement can be expected.

(3) Successful and unsuccessful foreign entities will be contacted directly by the NASA sponsoring office. Copies of these letters will be sent to the foreign sponsor. Should a foreign proposal or a U.S. proposal with foreign participation be selected, NASA's Office of External Relations will arrange with the foreign sponsor for the proposed participation on a no-exchange-of-funds basis, in which NASA and the foreign sponsor will each bear the cost of discharging their respective responsibilities.

(4) Depending on the nature and extent of the proposed cooperation, these arrangements may entail: (i) An exchange of letters between NASA and the foreign sponsor; or (ii) A formal Agency-to-Agency Memorandum of Understanding (MOU).

VI. EXPORT CONTROL GUIDELINES APPLICABLE TO FOREIGN PROPOSALS AND PROPOSALS INCLUDING FOREIGN PARTICIPATION.

Foreign proposals and proposals including foreign participation must include a section discussing compliance with U.S. export laws and regulations, e.g., 22 CFR Parts 120-130 and 15 CFR Parts 730-774, as applicable to the circumstances surrounding the particular foreign participation. The discussion must describe in detail the proposed foreign participation and is to include, but not be limited to, whether or not the foreign participation may require the prospective proposer to obtain the prior approval of the Department of State or the Department of Commerce via a technical assistance agreement or an export license, or whether a license exemption/exception may apply. If prior approvals via licenses are necessary, discuss whether the license has been applied for or if not, the projected timing of the application and any implications for the schedule. Information regarding U.S. export regulations is available at http://www.pmdtc.org and http://www.bxa.doc.gov. Proposers are advised that under U.S. law and regulations, spacecraft and their specifically designed, modified, or configured systems, components, and parts are generally considered "Defense Articles" on the United States Munitions List.

VII. EVALUATION AND SELECTION

A. EVALUATION PROCESS

The evaluation process for this NRA will begin with a scientific and technical peer review of the submitted proposals. NASA will also evaluate the proposed budget. For flight definition proposals, NASA will also conduct engineering and management reviews to establish feasibility of the planned implementation, to review risk mitigation plans, to evaluate the experience of the implementing organization, and to review the budget. The programmatic objectives of this NRA, as discussed in the introduction to this Appendix, will be applied by NASA to enhance program breadth, balance, and diversity. Upon completion of deliberations, offerors will be notified regarding proposal selection or rejection. Offerors whose proposals are declined will have the opportunity of a verbal debriefing with a NASA representative regarding the reasons for this decision. Additional information on the evaluation and selection process is given in Appendix C.

B. EVALUATION FACTORS

This section replaces Section I of Appendix C. The principal elements considered in the evaluation of proposals solicited by this NRA are relevance to NASA's objectives, intrinsic merit, and cost. Of these, intrinsic merit has the greatest weight, followed by relevance to NASA's objectives, of slightly lesser weight. Both of these elements have greater weight than cost. Evaluation of the intrinsic merit of the proposal includes consideration of the following factors, in descending order of importance:

- Overall scientific or technical merit, including evidence of unique or innovative methods, approaches, or concepts, and the potential for new discoveries or understanding, or delivery of new technologies/products;
- Qualifications, capabilities, and experience of the proposed Principal Investigator, team leader, or key personnel who are critical in achieving the proposal objectives;
- Institutional resources and experience that are critical in achieving the proposal objectives;
- Proposed plan for education and public outreach activities. Examples include such items as involvement of students in the research activities, technology transfer plans, public information programs that inform the general public of benefits gained from the research, and/or plans for incorporation of scientific results obtained into educational curricula.
- Overall standing among similar proposals available for evaluation and/or evaluation against the known state-of-the-art.

Evaluation of a proposal's relevance to NASA's objectives includes the consideration of the potential contribution of the effort to NASA's mission. Evaluation of the cost of a proposed effort includes

consideration of the realism and reasonableness of the proposed cost, and the relationship of the proposed cost to available funds.

The scientific peer review panel will assign each proposal a numerical merit score from 1 (worst) to 9 (best) based on the above factors. The score assigned by the peer review panel will not be affected by the cost of the proposed work nor will it reflect the programmatic relevance of the proposed work to NASA. However, the panel will be asked to include in their critique of each proposal any comments they may have concerning the proposal's budget and relevance to NASA. For flight proposals, the engineering and management panel will evaluate the feasibility and complexity of accomplishing each investigation along with an estimate of total cost for flight hardware development. NASA will take these assessments into consideration in making the final selection of experiments that will be considered for flight definition.

C. SELECTION CATEGORIES, PERIOD OF SUPPORT, AND FLIGHT-DEFINITION PROCESS

Proposals selected for support through this NRA will be selected as either ground-based or flight-definition investigations. Investigators offered support in the ground-based program normally will be required to submit a new proposal for competitive renewal after no more than four years of support. Investigators offered flight-definition status are expected to begin preparing experiment requirements and concepts for flight development shortly after selection in cooperation with the assigned representative from NASA. The selected investigations will be required to comply with MRD policies, including the return of all appropriate information for inclusion in the MRD archives during the performance of and at the completion of the contract or grant. Commitment by NASA to proceed from flight-definition to the execution of a flight experiment will be made only after several additional engineering and scientific reviews and project milestones have established the feasibility and merit of the proposed experiment. Investigations that do not pass these reviews will be funded for a limited period (generally four years from the initial award date) to allow an orderly termination of the project.
The Principal Investigator in flight-definition must prepare a Science Requirements Document (SRD) early in the development of a flight experiment to guide the design, engineering, and integration effort for the instrument. The SRD describes specific experiment parameters, conditions, background, and justification for flight. Ground-based experimentation may also be required to prepare an adequate SRD. The amount of support (technical, scientific, and budgetary) provided to investigators by NASA will be determined based upon specific investigator needs and the availability of resources. It should be noted that while a proposer can propose multiple flights, in general NASA will not commit to more than one flight without a reflight review after the first flight.

VIII. NRA FUNDING

The total amount of funding for this program is subject to the annual NASA budget cycle. The Government's obligation to make awards is contingent upon the availability of appropriated funds from which payment for award purposes can be made and the receipt of proposals which the Government determines are acceptable for an award under this NRA. For the purposes of budget planning in the Biotechnology Program, we have assumed that 5 to 8 flight experiment definition proposals will be funded. These efforts are typically funded at an average of $175,000 per year. It is also anticipated that approximately 32 ground-based investigations will be funded, at an average of approximately $140,000 per year, for up to 4 years. In addition, two to four interdisciplinary projects will be funded at an average cost of approximately $500,000 per year.

The initial fiscal year funding for all proposals will be adjusted, if required, to reflect partial fiscal year efforts.

The proposed budgets should include researcher's salary, travel to science and NASA meetings, other expenses (publication costs, computing or workstation costs), burdens, and overhead.

IX. BIBLIOGRAPHY

The *Microgravity Science and Applications Program Tasks and Bibliography for FY 1999*, which contains a searchable data base on currently funded research, may provide useful information to proposers and can be viewed at the following web location:
http://peer1.idi.usra.edu/peer_review/taskbook/taskbook.html

NRC Report on *Future Biotechnology Research on the International Space Station* is available at the following web location: www.nationalacademies.org/ssb/btfmenu.htm

HARDWARE AND FACILITY DESCRIPTIONS

CELLULAR AND MACROMOLECULAR BIOTECHNOLOGY

NASA's Microgravity Research Division (MRD) is currently pursuing a program for the development of payloads capable of accommodating multiple users. This program is expected to meet the science requirements of investigations and to support the development of technologies for biotechnology research. In the interest of minimizing project cost and complexity, NASA encourages the use of existing U.S. or Internationally developed space hardware whenever consistent with experiment requirements.

The equipment described is either planned for development or has been developed by NASA. International equipment described here may be offered for flight on a cooperative basis with agencies from various countries. The availability of the hardware described in this section is contingent upon the availability of funds, flight manifest opportunities, and, for international hardware, cooperative agreements between NASA and the appropriate foreign space agency.

I. CURRENT FLIGHT HARDWARE

The experimental hardware described in this section is available with or without modification contingent upon the availability and allocation of resources.

A. MIDDECK GLOVEBOX (MGBX) (NASA/MSFC)

The Middeck Glovebox is multi-user and multidiscipline facility that provides an enclosed working space for experiment manipulation and observation. Glovebox Investigations which have flown include: protein crystal growth, fluid physics, combustion, and materials science experiments.

The MGBX occupies two standard lockers in the Space Shuttle middeck. The MGBX door opening to insert or retrieve investigation hardware is 20.3 cm by 19.4 cm, with a working volume of 35 liters. Forced air cooling can withdraw a maximum of 60 W of investigation generated heat. Up to 60 W of 24, ± 12, and 5 VDC power is available for experimenter apparatus. The MGBX can be used in various modes of pressure and air circulation. The working area can serve as a sealed environment that is isolated from the crew cabin atmosphere, as a constantly recirculating atmosphere that is maintained at a pressure slightly lower that the middeck ambient, or as a working area open to the middeck. Multipurpose filters exist to remove particles, liquids, and reaction gasses from the recirculated air.

Due to limitations of the Space Shuttle middeck, there is no standard data or video downlink. There is the possibility of some near real-time video downlink (from the Shuttle Camcorder), but this will be decided on a mission-by-mission basis. Four video recorders provide data storage, with digital data stored in the audio channels; an additional connector records eight channels of data to the Interface frame data recorder. An adjustable light switch, video port plugs, a backlight panel, a halogen flashlight, redlight filters, and cutout window covers provide illumination.

B. THERMAL ENCLOSURES

Use of the Single-locker Thermal Enclosure System (STES) or the Thermal Enclosure System (TES) is encouraged for investigations requiring experiment temperature control. Because the STES and TES are frequently manifested in the middeck of the Shuttle Orbiter, the equipment can be installed shortly before a launch. Current timetables provide late access at approximately 24 hours before launch and early removal at three-to-eight hours after Shuttle landing. A thermal enclosure to meet the more demanding requirements of payloads on the International Space Station is under development. Ideally, equipment built to be accommodated in the TES or STES should be fully automated. However, some crew time may be available for tending of experiments (e.g., activation or deactivation of protein crystal growth process)

on some flights. The proposer should recognize that crew time and communication channels are subject to mission priorities that place experiment management behind crew health, safety concerns, and accomplishment of the primary mission.

1. Single Locker Thermal Enclosure System (STES)
   The STES is the size of a single middeck or Spacehab locker and provides a controlled temperature environment within plus or minus 0.5° C of a set point in the range from 4 to 40° C; the set point must be within 24° C of ambient temperature. The STES time/temperature profile is programmable within the thermal capability of the hardware. Internal STES heat is transferred primarily by conduction. The STES has nine sensors which are placed at various locations to record temperature history and to provide temperature control. Temperature data is available post-mission. A payload assembly, consisting of an STES and an experiment apparatus, must meet interface, operational, and safety requirements of the vehicle or space platform used. A small amount of power is available for use by an experiment apparatus; use of this resource may impact the temperature control capability of the unit. Experiment duration, available crew time, and air-to-ground communication capability for STES payloads is mission dependent. Periodic monitoring of STES operation is required. The STES door can be opened easily to accommodate experiment operations.

2. Thermal Enclosure System (TES) The TES is the size of two vertically adjacent middeck lockers and provides a controlled temperature environment within plus or minus 0.2° C of a set point in the range from 4 to 40° C; the set point must be within 24° C of ambient temperature. The TES time/temperature profile is programmable within the thermal capability of the hardware. Internal TES heat is transferred primarily by convection. The TES has nine sensors which are placed at various locations to record temperature history and to provide temperature control. Temperature data is available post-mission. A payload assembly, consisting of a TES and an experiment apparatus, must meet interface, operational, and safety requirements of the vehicle or space platform used. A small amount of power is available for use by an experiment apparatus; use of this resource may impact the temperature control capability of the unit. Experiment duration, available crew time, and air-to-ground communication capability for TES payloads is mission dependent. Periodic monitoring of TES operation is required. The TES door is not normally removed for experiment operations: mechanical and electrical/electronic functions are accomplished by use of "feed throughs" in the TES door. An experiment-unique TES door may be proposed.

C. PROTEIN CRYSTAL GROWTH HARDWARE

1. Protein Crystallization Apparatus for Microgravity (PCAM)
   The PCAM is a protein crystal growth device that has been developed to provide a large number of protein crystallization experiments in a single middeck locker flight. Six cylindrical PCAM units can be mounted in an STES unit. Each cylindrical PCAM contains nine crystallization plates, each having seven sample chambers, for a total of 63 chambers per cylinder. Thus, the total number of samples that can be flown in an STES is 378. The crystallization plates are modified "sitting drop" vapor diffusion devices based on the commercial CrysChem design. In the center of each chamber is a pedestal with a depression on its top which can contain up to 40 microliters of pre-mixed protein sample solution and precipitant solution. The pedestal is surrounded by a toroidal reservoir of absorbent material capable of containing approximately one milliliter of precipitant solution. The protein solution is isolated from the reservoir prior to activation and after deactivation. Activation occurs simultaneously for all chambers in a cylinder, as does deactivation. PCAMs can also be flown in protective bags at ambient temperature when samples do not require +/- .5 C temperature control.

2. Diffusion-controlled Protein Crystallization Apparatus for Microgravity (DCAM)
   The DCAM hardware, which was designed for long duration protein crystal growth on the Mir Space Station, combines liquid-liquid diffusion and dialysis methods to effect protein crystal growth. Each DCAM tray assembly consists of 27 DCAM experiment chambers containing precipitant solutions and protein sample solutions. These chambers are arranged in nine rows of three units each and are mounted on an aluminum tray. No crew activation or deactivation of the hardware is required: the DCAM does not employ mechanical activation/deactivation. Crystallization conditions are approached very slowly. Each DCAM self-activates as the precipitant solution slowly diffuses through a control plug from a larger solution chamber into and across a smaller solution chamber until it reaches a small volume of protein sample solution separated from the chamber by a dialysis membrane.

3. Protein Crystallization Facility - Light Scattering Temperature (PCF-LST)
   The PCF-LST grows crystals using the same temperature induced growth process as the PCF. The PCF-LST incorporates a laser light scattering device that detects nucleation and displays the corresponding detector voltage level on a Macintosh Powerbook. This display allows a crew member to identify when nucleation has occurred in the sample and to adjust the temperature profile accordingly to control the growth period. This experiment hardware accommodates two sample bottles up to 50 milliliters in size, in one thermal enclosure. In addition, approximately one standard middeck locker of stowage is required for support equipment.

4. Protein Crystallization Facility-Variable Gradient (PCF-VG)
   This equipment is used to allow customers to process small amounts of protein using the temperature induced crystal growth process. Sample sizes for the PCF-VG include 1 ml and 5 ml. By varying thermal path configurations internal to the thermal enclosure used, investigators can obtain several temperature profiles for different samples. By allowing customers to screen a large number of conditions on a single flight, users are able to investigate a variety of growth parameters.

D. CELLULAR BIOTECHNOLOGY SYSTEMS

The Cellular Biotechnology flight hardware provides the technological capability for addressing the potential of microgravity as a tool in tissue engineering and cell science. Major flight experiment equipment for Cellular Biotechnology includes the following: Rotating Wall Perfused Systems (RWPS), an incubator called the Biotechnology Specimen Temperature Controller (BSTC), Experiment Control Computer (ECC), Gas Supply Module (GSM), and the Biotechnology Refrigerator (BTR). The Cellular Biotechnology flight hardware offers increased levels of tissue culturing capability and automation allowing investigators to culture cells and tissues under the low mechanical shear environment of microgravity. The flight hardware and ground-based rotating wall culture vessels suspend cells and tissue about a rotating horizontal axis creating a low fluid shear environment, and have successfully cultured suspension and anchorage-dependent mammalian cells. The vessels include features that allow addition of nutrients, removal of metabolic waste products, respiratory gas exchange, temperature control, and sample removal.

1. Slow Turning Lateral Vessel (STLV)
   The STLV is a nonperfused, horizontally rotating bioreactor consisting of a fixed volume vessel (50 or 100 ml). The vessel is connected to a variable-rate motor and mounted on a fixed base. The STLV is autoclavable. The vessel has several separate sample ports for adding media or reagents and removing samples. The STLV has been optimized for to culture anchorage-dependent cells on microcarrier systems. It is commercially available through Synthecon, Inc., Houston, TX.

2. High Aspect Ratio Vessel (HARV)
   The HARV is a nonperfused, horizontally rotating bioreactor consisting of a fixed volume vessel (10 or 50 ml) with a large radius and a short length. The vessel is connected to a variable-rate motor and mounted on a fixed base. The HARV is autoclavable. The vessel has several separate sample ports for adding media or reagents and removing of samples. The HARV has been optimized to culture suspension cells and anchorage-dependent cells with or without microcarriers. It is commercially available through Synthecon, Inc., Houston, TX.

3. The Hydrodynamic Focusing Bioreactor (HFB)
   The Hydrodynamic Focusing Bioreactor model HFB-EM2, Celdyne, Inc., Houston, TX, is supplied with a 150 ml culture vessel and a differential spinner drive to facilitate positional control of cells and aggregates within the vessel. The vessel rotation rate can be set with crystal controlled accuracy from 1 to 30 RPM in 1 RPM increments. The spinner rotation rate is similarly adjustable from 1 to 99 RPM. This unit is functionally identical to those now in use by the NASA Cellular Biotechnology Program at the Johnson Space Center. The perfusion coupling kit contains a rotating fluid coupler and an extended mounting base which provides the fluid coupling interfaces required to implement a perfusion/infusion system.

   The HFB provides a dramatic reduction in shear force exerted on cell aggregates in culture compared to other systems. Early testing has shown this characteristic produces larger cell aggregates, which provides the potential for advances in your field of research. The HFB incorporates an internal viscous spinner that may be rotated at varying speeds relative to the vessel dome. This differential motion produces a hydrodynamic focusing effect that is unique to the HFB. The resulting force suspends cells in a low-shear environment (0.01 dyne/cm2 maximum @ 10 rpm) that supports the formation of delicate assemblies. The magnitude of the effect can be controlled by adjusting the differential rotation rate of the spinner and vessel, thus providing direct control over the location of cells, tissues and bubbles within the vessel. This controllability facilitates culture sampling and bubble removal without degrading the low shear environment required to culture delicate tissue aggregates.

4. The Rotating Wall Perfused Vessel (RWPV)
   The RWPV is a perfused, horizontally rotating bioreactor consisting of a fixed volume vessel (250 or 500 ml), a silicone membrane oxygenator, a pH sensor, sample ports, and a pump for infusing or recycling fresh medium. The RWPV is sterilized with ethylene oxide in a specially designed apparatus. The vessel is secured to a support base and connected to two variable-rate motors that independently control the rotation of the vessel's outer wall and the hollow inner centerline spin filter. Rotation rates for the vessel's outer wall and spin filter can be varied in order to create different levels of fluid shear and turbulence. Samples are withdrawn through sample ports; the vessel's outer wall can be stopped temporarily during sampling. Fresh or recycled media can be perfused into the vessel at rates sufficient to support nutrient delivery, metabolic gas exchange, and waste-product removal. A version of the RWPV has been used to transition cell cultures to microgravity. It is commercially available through Synthecon, Inc., Houston, TX. Flight hardware is available through the Cellular Biotechnology Program.

5. The Biotechnology Specimen Temperature Controller (BSTC)
   The BSTC is flight equipment capable of transporting and maintaining biological and cell culture samples in a controlled temperature. The BSTC is a self-contained unit that can maintain a variety of specimen volumes up to 50 ml at temperatures from ambient to 37°C. The device can maintain target temperatures in this range during launch, mission and reentry. In its current format the BSTC can accommodate 32 (15 ml) or 64 (7ml) cell cultures. The BSTC is a flight incubator and is used for the definition phase of the experiment protocol and for fundamental cell biology experiments investigating the events leading to tissue morphogenesis.

6. The Biotechnology Refrigerator (BTR)

The BTR is a 0.6 cu ft cold storage unit that maintains 4°C for maintenance of specimens, media, and reagents. It is thermoelectrically driven by a series of Peltier diodes.

7. Cryodewar
The GN2 Freezer is a Commercial Off The Shelf (COTS) Cellular Biotechnology Cryodewar (CBC) with minor modifications that will be used to transport cryo samples from earth to the International Space Station. The dewar has a 0.9 liter specimen volume and retains cryogenic temperature for up to 20 days.

8. The Cell Culture Unit (CCU) Ames Research Center

It is possible to fly live cell cultures of various types for up to 90 days on orbit. Types of cultures that can be used include suspended animal, microbial, and plant cultures, attachment cultures, tissues, and small, non-feeding aquatic organisms. Cell cultures of 3, 10, or 30 ml volume can be maintained within a temperature range of 4 to 39°C, and an atmosphere with controlled temperature, pH, carbon dioxide, and oxygen levels. Cell images can be observed and evaluated in situ using 40 or 200x total optical magnification differential image contrast or brightfield microscopy, or cell chambers can be removed for imaging using a phase-contrast/fluorescence microscope with higher magnification. Images may be transmitted to the ground laboratories when required. The experiment may be designed with simultaneous onboard reference samples under an artificial gravity (0.001 to 2.0g) environment. Nutrients or special additives can be introduced into the culture media automatically, and waste products can be removed automatically to maintain a specific growing environment. In addition, fixatives may be introduced to terminate a study and prepare specimens for further analysis on the ground. Alternatively, specimens or the culture medium may be sampled on orbit directly for further manipulation or storage. Simple cell manipulation essential for the experiment, such as solution mixing, DNA/RNA extraction, trypsinization, filtration, and concentration, may be carried out by a semi-automated method or by assistance of the crew. Videomicroscopy of either 40 or 200x, spectrophometry, and a phase-contrast/fluorescence microscope will be provided. The culture chamber environment is sterile, and cells may be placed on the spacecraft just prior to launch.

Hardware to support research on cells	Shuttle-Based	ISS-based	Agency
Cell Culture Module – no centrifuge capacity	X	X	NASA
DLR SIMPLEX	X	X	DLR
Biopack	X		ESA
Cell Culture Unit		X	NASA
Cell Biology Experiment Facility		X	NASDA
Modular Cultivation System		X	ESA
Incubator		X	NASA
Biolab		X	ESA

E. INTERNATIONAL BIOTECHNOLOGY EQUIPMENT

Protein Crystallization Diagnostics Facility (PCDF) (European Space Agency, ESA)
The PCDF is a multi-user experimental facility capable of providing in-depth knowledge and understanding of protein crystal growth processes under microgravity. Four experiment assemblies will be accommodated in the process chamber each consisting of a reactor with the protein and salt solutions with liquid sample volumes in the range of 50 ul to 6 ml, the associated drive system to activate and control the process, and the thermal control elements, which allow individual temperature control of each protein experiment. The PCDF will perform protein crystallization by dialysis method where the protein

solution and the salt solution are separated by a semi-permeable membrane, and by batch method where the saturation to get crystals is achieved by changing the temperature. The scientific samples in the Reactors will be monitored by some or all of the following:

a.) High-resolution black and white video system
b.) Video microscope
c.) Light scattering, Mach-Zehnder Phase Shift interferometer.

Each Reactor is temperature controllable for a range of 6 to 30° C. The PCDF is designed to fly in the European Drawer Rack (1 Middeck Locker and 1 Drawer Equivalents) on the International Space Station.

F. OTHER BIOTECHNOLOGY FLIGHT HARDWARE

1. Commercial Generic Bioprocessing Apparatus (CGBA)
The CGBA payload consists of a combination of temperature controlled locker replacement modules and fluid containment/mixing devices. It is compatible with Shuttle middeck, Spacehab, Spacelab and Mir interface requirements. Preparation of the CGBA payload allows samples to be loaded off-site and shipped to KSC for final integration, if so desired. Late access handover (L-24 hours) minimizes the time required between loading and launch for viable samples. Established protocol provides the opportunity to perform synchronized ground controls in flight-like hardware. Clinorotation in flight hardware is also available. The payload has flown on 8 STS missions since 1992 returning over 2000 cumulative biological and material samples at a better than 99% success ratio.. Experiments supported by CGBA have included: microorganism growth, eucaryotic cell response, virus capsid formation, crystal growth, collagen and fibrin polymerization, and mammalian tissue development. The individual components of CGBA are described below:

a) Fluid Processing Apparatus (FPA)
An FPA is essentially a "microgravity test tube". The first level of sample containment consists of a glass barrel (1.35 cm id x 11.7 cm) with movable rubber septa used to confine the fluids in separate chambers within the barrel. All components contacting the sample material are fully autoclavable allowing sterility to be maintained. The design provides initial isolation of 2 or 3 fluids and allows subsequent, on-orbit mixing. Fluid mixing is achieved as the fluid and septa are pushed forward until the fluid reaches a molded bypass in the glass barrel and flows around the forward septum into the adjacent chamber. The standard configuration provides a total liquid volume of 6.5 ml loaded as follows: 1.5 ml fixative / 1.5 ml initiator / 3.5 ml precursor. A sealed, Lexan sheath with a plunger handle encompasses the glass barrel providing an activation mechanism and a second level of containment. A positive pressure integrity test to 5 psi is performed on this preflight. Visual observation of samples in an FPA is possible and in-flight video or still photographs can be obtained.

Many variations of fluid volumes and configurations are possible. Several examples of modified FPAs include:

(i) Gas Exchange-FPA (GE-FPA): The GE-FPA has a gas permeable endcap and an O-ring Lexan insert is used in place of the distal rubber septum, thus allowing gas exchange between sample and entire GAP volume.

(ii) Expanded Volume-FPA (EV-FPA): Provides up to 10 ml into 1 ml mixing capability.

b) Group Activation Pack (GAP)
The GAP provides a third level of fluid containment composed of Lexan and aluminum. It allows simultaneous activation of 8 FPAs through attachment of a manual crank handle to a drive mechanism. (Used in GBA-INC or can be stowed in an ambient locker). A positive pressure integrity test to 5 psi is also performed on the GAPs preflight. Auto-GAP is activated automatically by an external DC motor drive rather than a manual crank.

c) GBA-INC
The GBA-INC is a middeck locker equivalent providing stowage for 9 GAPs (72 FPAs) at 37° C. Uniform temperature control is achieved using top and bottom strip heaters thermally coupled to the GAP aluminum endcaps. Optical density (565 nm) monitoring capability of 8 FPAs concurrently allows high resolution reaction rate data to be collected real-time.

d) GBA-ICM
GBA-ICM is a middeck locker equivalent which provides temperature controlled stowage for 8 GAPs (64 FPAs) adjustable between 4° C and 37° C. Thermoelectric modules are used to transfer heat to/from active water loops distributed around all 6 sides to virtually eliminate thermal gradients. An accelerometer-based system is used to detect launch, thus allowing the GAPs to begin initiating experiments immediately upon entering orbit. Additionally, automatic GAP determination can be programmed to occur at any time during the mission, including just prior to reentry based on preplanned (or updated) end of mission time. Combined, these two capabilities allow an early-as-possible experiment initiation and a late-as-possible termination; periods when crew availability for manual tasks is at a minimum. GBA-ICM also provides control versatility in light of launch delays, and can take advantage of mission duration extensions.

2. ADvanced SEPerations (ADSEP).
ADSEP is a fully-automated, processing unit that fits into a middeck or Spacehab locker. It is capable of separating living cells and cellular organelles using aqueous two-phase partitioning. The flight hardware contains three independently controlled processing modules, which can be programmed for totally automated operation or controlled via telemetry. Processing temperature can be independently controlled and regulated between 4-40° C in each of the three processing modules. Biological samples are loaded into a liquid-tight cassette assembly, which allows the cassettes to be installed and removed from the ADSEP modules on orbit. Processing consists of mixing with a programmable electromagnetic stirring system, and indexing the sample storage plates countercurrently. Each sample can be processed through up to 22 stages, employing a wide range of mixing, separation, and indexing parameters. In addition to separating cells, ADSEP has been employed for other fluids experiments where mechanical agitation, electromagnetic fields, and/or transfer of liquids from one well to the next is desired.

3. Materials Dispersion Apparatus (MDA)
Minilab The MDA is an automated laboratory which conducts approximately 100 fluid experiments within a brick-sized volume. Experiments which have been successfully conducted with this hardware include protein and other crystal growth, microencapsulation, thin film membrane formation, live cell culture studies, collagen formation, seed germination, and fluid science research. The heart of the MDA consists of a pair of blocks containing dozens of small test-tube like volumes of 20 to 500 ul each. Once in microgravity, the blocks are moved and the fluids which were separated are brought together to mix by either liquid-liquid diffusion, vapor diffusion, turbulent mixing, or wetting, depending upon the experiment design. As an option, the two liquids can be separated at a later time, or a third can be mixed in, such as a fixative for a cell culture experiment.

The MDA Minilab has to date successfully processed hundreds of biotechnology and other samples on the Shuttle. Four of the MDA Minilabs can be placed within a temperature-controlled middeck locker, for a total of up to 400 samples per locker. The MDA has flown on six Shuttle missions, along with six sounding rocket flights and the KC-135 low-g aircraft.

G. FLIGHT HARDWARE UNDER DEVELOPMENT

1. Space Station Thermal Enclosure Systems
A modified version of the STES is nearing completion for use on early International Space Station (ISS) flights until UF-3. These STES units will fly in an Express Rack. These units can be remotely

operated and have the capability of downloading data through an RS-422 port on the front panel of the STES.

Beginning with UF-3 a new fleet of single-locker thermal enclosure systems will be available for use on the International Space Station. These units are designed to utilize the ISS avionics air system. They will have a longer operating life, can be commanded from the ground, and will have data downloading capabilities. The units will also accommodate existing hardware designed to fit inside an STES. Temperature ranges and accuracies are expected to be slightly better than an STES (goal is 4-50 DegC, +/- 0.25 DegC accuracy).

2. Interferometer for Protein Crystal Growth (IPCG)
The IPCG, which was originally designed as an experiment to be operated in the Mir Glovebox, comprises three major systems -- an interferometer, six fluid assemblies with test cells, and a flight data system. The IPCG interferometer system employs a Michelson-Morley phase-shift interferometer to produce images showing density changes in the solution as a protein crystal forms. The IPCG crystal growth cells are made of optical grade glass: Cells are 1 mm thick and contain 250 ul of solution. Each fluid handling system is a self-contained plastic assembly enclosing two pairs of 4-ml supply syringes (one containing protein solution and one containing precipitant solution), a waste receptacle, and a test cell -- plus mechanisms to inject fluids and to position the test cell. The crew operates the fluid system with a hand crank that depresses the syringe pistons. The flight data system includes a 486-based laptop computer and has video recording capability. A modified version of the apparatus that flew on Mir is under development to make the unit compatible with ISS requirements and to improve the robustness of the fluid systems.

II. GROUND-BASED FACILITIES

Investigators often need to conduct reduced gravity experiments in ground-based facilities during the experiment definition and technology development phases. The NASA ground-based reduced gravity research facilities that support the MRD program include two drop towers at the Glenn Research Center (GRC), a DC-9 and KC-135 aircraft.

A. 2.2-SECOND DROP TOWER (NASA, Glenn Research Center)

The 2.2-Second Drop Tower at GRC provides 2.2 second of low-gravity test time for experiment packages consisting of up to 125 kilograms of hardware. The experiment package is enclosed in a drag shield and a gravitational acceleration of less than 10^{-5}g is obtained during the fall since the experiment package falls freely within the drag shield. At the end of a drop, the drag shield and the enclosed experiment are decelerated in a 2.2-meter deep sand pit by the deceleration spikes. The peak deceleration rate can be as high as 70g's. Eight to twelve tests can be performed in one day. Data from experiments are acquired by high-speed motion picture cameras with rates up to 1,000 frames per second and by onboard data acquisition systems used to record data supplied by thermocouples, pressure transducers, and flow meters.

B. 5.18-SECOND ZERO-GRAVITY FACILITY (NASA, Glenn Research Center)

The 5.18-second Zero-Gravity facility at GRC has a 132-meter free fall distance in a drop chamber which is evacuated by a series of pumpdown procedures to a final pressure of 1 Pa. Experiments with hardware weighing of up to 450 kilograms are mounted in a one-meter diameter by 3.4-meter high drop bus. Gravitational acceleration of less than 10^{-5} is obtained. At the end of the drop, the bus is decelerated in a 6.1-meter deep container filled with small pellets of expanded polystyrene. The deceleration rate is typically 60g (for 20 millisec). Visual data is acquired through the use of high-speed motion picture cameras. Also, other data such as pressures, temperatures, and accelerations are either recorded onboard with various data acquisition systems or are transmitted to a control room by a telemetry system capable of transmitting 18 channels of continuous data. Due to the complexity of drop chamber operations and time required for pump-down of the drop chamber, typically only one test is performed per day.

C. PARABOLIC AIRCRAFT (NASA)

The aircraft can provide up to 40 periods of low-gravity for 25-second intervals each during one flight. The aircraft accommodates a variety of experiments and is often used to refine space flight experiment equipment and techniques and to train crew members in experiment procedures, thus giving investigators and crew members valuable experience working in a weightless environment. The aircraft obtain a low-gravity environment by flying a parabolic trajectory. Gravity levels twice those of normal gravity occur during the initial and final portions of the trajectory, while the brief pushover at the top of the parabola produces less than one percent of Earth's gravity (10^{-2}g). Several experiments, including a combination of attached and free-floated hardware (which can provide effective gravity levels of 10^{-3} for periods up to 10 seconds) can be integrated in a single flight. Both 28-volt DC and 100-volt AC power are available. Instrumentation and data collection capabilities must be contained in the experiment packages.

III. COMPUTATIONAL SUPPORT AND DATA MANAGEMENT

NASA provides an advanced computational environment incorporating supercomputers, high performance mass storage, and software. NASA also provides an on-line, multidisciplinary directory of space science data sets of interest to the NASA-sponsored research community. NASA has chartered the NASA Science Internet (NSI) to provide transparent wide-area network connectivity to NASA researchers, computational resources, and data, worldwide. Each of these facilities and resources should be considered by an investigator to determine which are required for conducting biotechnology research. Investigators should include any requirements for theses resources in their proposal.

INSTRUCTIONS FOR RESPONDING TO NASA RESEARCH ANNOUNCEMENTS

(SEPTEMBER 1999)

A. General.

(1) Proposals received in response to a NASA Research Announcement (NRA) will be used only for evaluation purposes. NASA does not allow a proposal, the contents of which are not available without restriction from another source, or any unique ideas submitted in response to an NRA to be used as the basis of a solicitation or in negotiation with other organizations, nor is a pre-award synopsis published for individual proposals.

(2) A solicited proposal that results in a NASA award becomes part of the record of that transaction and may be available to the public on specific request; however, information or material that NASA and the awardee mutually agree to be of a privileged nature will be held in confidence to the extent permitted by law, including the Freedom of Information Act.

(3) NRAs contain programmatic information and certain requirements which apply only to proposals prepared in response to that particular announcement. These instructions contain the general proposal preparation information which applies to responses to all NRAs.

(4) A contract, grant, cooperative agreement, or other agreement may be used to accomplish an effort funded in response to an NRA. NASA will determine the appropriate instrument. Contracts resulting from NRAs are subject to the Federal Acquisition Regulation and the NASA FAR Supplement. Any resultant grants or cooperative agreements will be awarded and administered in accordance with the NASA Grant and Cooperative Agreement Handbook (NPG 5800.1).

(5) NASA does not have mandatory forms or formats for responses to NRAs; however, it is requested that proposals conform to the guidelines in these instructions. NASA may accept proposals without discussion; hence, proposals should initially be as complete as possible and be submitted on the proposers' most favorable terms.

(6) To be considered for award, a submission must, at a minimum, present a specific project within the areas delineated by the NRA; contain sufficient technical and cost information to permit a meaningful evaluation; be signed by an official authorized to legally bind the submitting organization; not merely offer to perform standard services or to just provide computer facilities or services; and not significantly duplicate a more specific current or pending NASA solicitation.

B. NRA-Specific Items. Several proposal submission items appear in the NRA itself: the unique NRA identifier; when to submit proposals; where to send proposals; number of copies required; and sources for more information. Items included in these instructions may be supplemented by the NRA.

C. Proposal Content. The following information is needed to permit consideration in an objective manner. NRAs will generally specify topics for which additional information or greater detail is desirable. Each proposal copy shall contain all submitted material, including a copy of the transmittal letter if it contains substantive information.

(1) *Transmittal Letter or Prefatory Material*.

(i) The legal name and address of the organization and specific division or campus identification if part of a larger organization;

(ii) A brief, scientifically valid project title intelligible to a scientifically literate reader and suitable for use in the public press;

(iii) Type of organization: e.g., profit, nonprofit, educational, small business, minority, women-owned, etc.;

(iv) Name and telephone number of the principal investigator and business personnel who may be contacted during evaluation or negotiation;

(v) Identification of other organizations that are currently evaluating a proposal for the same efforts;

(vi) Identification of the NRA, by number and title, to which the proposal is responding;

(vii) Dollar amount requested, desired starting date, and duration of project;

(viii) Date of submission; and

(ix) Signature of a responsible official or authorized representative of the organization, or any other person authorized to legally bind the organization (unless the signature appears on the proposal itself).

(2) *Restriction on Use and Disclosure of Proposal Information*. Information contained in proposals is used for evaluation purposes only. Offerors or quoters should, in order to maximize protection of trade secrets or other information that is confidential or privileged, place the following notice on the title page of the proposal and specify the information subject to the notice by inserting an appropriate identification in the notice. In any event, information contained in proposals will be protected to the extent permitted by law, but NASA assumes no liability for use and disclosure of information not made subject to the notice.

(3) *Abstract*. Include a concise (200-300 word if not otherwise specified in the NRA) abstract describing the objective and the method of approach.

(4) *Project Description*.

(i) The main body of the proposal shall be a detailed statement of the work to be undertaken and should include objectives and expected significance; relation to the present state of knowledge; and relation to previous work done on the project and to related work in progress elsewhere. The statement should outline the plan of work, including the broad design of experiments to be undertaken and a description of experimental methods and procedures. The project description should address the evaluation factors in these instructions and any specific factors in the NRA. Any substantial collaboration with individuals not referred to in the budget or use of consultants should be described. Subcontracting significant portions of a research project is discouraged.

(ii) When it is expected that the effort will require more than one year, the proposal should cover the complete project to the extent that it can be reasonably anticipated. Principal emphasis should be on the first year of work, and the description should distinguish clearly between the first year's work and work planned for subsequent years..

(5) *Management Approach*.. For large or complex efforts involving interactions among numerous individuals or other organizations, plans for distribution of responsibilities and arrangements for ensuring a coordinated effort should be described.

(6) *Personnel*. The principal investigator is responsible for supervision of the work and participates in the conduct of the research regardless of whether or not compensated under the award. A short biographical sketch of the principal investigator, a list of principal publications and any exceptional qualifications should be included. Omit social security number and other personal items which do not merit consideration in evaluation of the proposal. Give similar biographical information on other senior professional personnel who will be directly associated with the project. Give the names and titles of any other scientists and technical personnel associated substantially with the project in an advisory capacity. Universities should list the approximate number of students or other assistants, together with information as to their level of academic attainment. Any special industry-university cooperative arrangements should be described.

(7) *Facilities and Equipment.*

(i) Describe available facilities and major items of equipment especially adapted or suited to the proposed project, and any additional major equipment that will be required. Identify any Government-owned facilities, industrial plant equipment, or special tooling that are proposed for use. Include evidence of its availability and the cognizant Government points of contact.

(ii) Before requesting a major item of capital equipment, the proposer should determine if sharing or loan of equipment already within the organization is a feasible alternative. Where such arrangements cannot be made, the proposal should so state. The need for items that typically can be used for research and non-research purposes should be explained..

(8) *Proposed Costs (U.S. Proposals Only).*

(i) Proposals should contain cost and technical parts in one volume: do not use separate "confidential" salary pages. As applicable, include separate cost estimates for salaries and wages; fringe benefits; equipment; expendable materials and supplies; services; domestic and foreign travel; ADP expenses; publication or page charges; consultants; subcontracts; other miscellaneous identifiable direct costs; and indirect costs. List salaries and wages in appropriate organizational categories (e.g., principal investigator, other scientific and engineering professionals, graduate students, research assistants, and technicians and other non-professional personnel). Estimate all staffing data in terms of staff-months or fractions of full-time.

(ii) Explanatory notes should accompany the cost proposal to provide identification and estimated cost of major capital equipment items to be acquired; purpose and estimated number and lengths of trips planned; basis for indirect cost computation (including date of most recent negotiation and cognizant agency); and clarification of other items in the cost proposal that are not self-evident. List estimated expenses as yearly requirements by major work phases.

(iii) Allowable costs are governed by FAR Part 31 and the NASA FAR Supplement Part 1831 (and OMB Circulars A-21 for educational institutions and A-122 for nonprofit organizations).

(iv) Use of NASA funds--NASA funding may not be used for foreign research efforts at any level, whether as a collaborator or a subcontract. The direct purchase of supplies and/or services, which do not constitute research, from non-U.S. sources by U.S. award recipients is permitted. Additionally, in accordance with the National Space Transportation Policy, use of a non-U.S. manufactured launch vehicle is permitted only on a no-exchange-of-funds basis.

(9) *Security.* Proposals should not contain security classified material. If the research requires access to or may generate security classified information, the submitter will be required to comply with Government security regulations.

(10) *Current Support.* For other current projects being conducted by the principal investigator, provide title of project, sponsoring agency, and ending date.

(11) *Special Matters*.

(i) Include any required statements of environmental impact of the research, human subject or animal care provisions, conflict of interest, or on such other topics as may be required by the nature of the effort and current statutes, executive orders, or other current Government-wide guidelines.

(ii) Proposers should include a brief description of the organization, its facilities, and previous work experience in the field of the proposal. Identify the cognizant Government audit agency, inspection agency, and administrative contracting officer, when applicable.

D. Renewal Proposals

(1) Renewal proposals for existing awards will be considered in the same manner as proposals for new endeavors. A renewal proposal should not repeat all of the information that was in the original proposal. The renewal proposal should refer to its predecessor, update the parts that are no longer current, and indicate what elements of the research are expected to be covered during the period for which support is desired. A description of any significant findings since the most recent progress report should be included. The renewal proposal should treat, in reasonable detail, the plans for the next period, contain a cost estimate, and otherwise adhere to these instructions.

(2) NASA may renew an effort either through amendment of an existing contract or by a new award.

E. Length.. Unless otherwise specified in the NRA, effort should be made to keep proposals as brief as possible, concentrating on substantive material. Few proposals need exceed 15-20 pages. Necessary detailed information, such as reprints, should be included as attachments. A complete set of attachments is necessary for each copy of the proposal. As proposals are not returned, avoid use of "one-of-a-kind" attachments.

F. Joint Proposals.

(1) Where multiple organizations are involved, the proposal may be submitted by only one of them. It should clearly describe the role to be played by the other organizations and indicate the legal and managerial arrangements contemplated. In other instances, simultaneous submission of related proposals from each organization might be appropriate, in which case parallel awards would be made.

(2) Where a project of a cooperative nature with NASA is contemplated, describe the contributions expected from any participating NASA investigator and agency facilities or equipment which may be required. The proposal must be confined only to that which the proposing organization can commit itself. "Joint" proposals which specify the internal arrangements NASA will actually make are not acceptable as a means of establishing an agency commitment.

G. Late Proposals.. Proposals or proposal modifications received after the latest date specified for receipt may be considered if a significant reduction in cost to the Government is probable or if there are significant technical advantages, as compared with proposals previously received.

H. Withdrawal. Proposals may be withdrawn by the proposer at any time before award. Offerors are requested to notify NASA if the proposal is funded by another organization or of other changed circumstances which dictate termination of evaluation.

I. Evaluation Factors.

(1) Unless otherwise specified in the NRA, the principal elements (of approximately equal weight) considered in evaluating a proposal are its relevance to NASA's objectives, intrinsic merit, and cost.

(2) Evaluation of a proposal's relevance to NASA's objectives includes the consideration of the potential contribution of the effort to NASA's mission.

(3) Evaluation of its intrinsic merit includes the consideration of the following factors of equal importance:

(i) Overall scientific or technical merit of the proposal or unique and innovative methods, approaches, or concepts demonstrated by the proposal.

(ii) Offeror's capabilities, related experience, facilities, techniques, or unique combinations of these which are integral factors for achieving the proposal objectives.

(iii) The qualifications, capabilities, and experience of the proposed principal investigator, team leader, or key personnel critical in achieving the proposal objectives.

(iv) Overall standing among similar proposals and/or evaluation against the state-of-the-art.

(4) Evaluation of the cost of a proposed effort may include the realism and reasonableness of the proposed cost and available funds.

J. Evaluation Techniques.. Selection decisions will be made following peer and/or scientific review of the proposals. Several evaluation techniques are regularly used within NASA. In all cases proposals are subject to scientific review by discipline specialists in the area of the proposal. Some proposals are reviewed entirely in-house, others are evaluated by a combination of in-house and selected external reviewers, while yet others are subject to the full external peer review technique (with due regard for conflict-of-interest and protection of proposal information), such as by mail or through assembled panels. The final decisions are made by a NASA selecting official. A proposal which is scientifically and programmatically meritorious, but not selected for award during its initial review, may be included in subsequent reviews unless the proposer requests otherwise.

K. Selection for Award.

(1) When a proposal is not selected for award, the proposer will be notified. NASA will explain generally why the proposal was not selected. Proposers desiring additional information may contact the selecting official who will arrange a debriefing.

(2) When a proposal is selected for award, negotiation and award will be handled by the procurement office in the funding installation. The proposal is used as the basis for negotiation. The contracting officer may request certain business data and may forward a model award instrument and other information pertinent to negotiation.

L. Additional Guidelines Applicable to Foreign Proposals and Proposals Including Foreign Participation.

(1) NASA welcomes proposals from outside the U.S. However, foreign entities are generally not eligible for funding from NASA. Therefore, [unless otherwise noted in the NRA] proposals from foreign entities should not include a cost plan unless the proposal involves collaboration with a U.S. institution, in which case a cost plan for only the participation of the U.S. entity must be included. Proposals from foreign entities and proposals from U.S. entities that include foreign participation must be endorsed by the respective government agency or funding/sponsoring institution in the country from which the foreign entity is proposing. Such endorsement should indicate that the proposal merits careful consideration by NASA, and if the proposal is selected, sufficient funds will be made available to undertake the activity as proposed.

(2) All foreign proposals must be typewritten in English and comply with all other submission requirements stated in the NRA. All foreign proposals will undergo the same evaluation and selection process as those originating in the U.S. All proposals must be received before the established closing date. Those received after the closing date will be treated in accordance with paragraph (g) of this provision. Sponsoring foreign government agencies or funding institutions may, in exceptional situations, forward a proposal without endorsement if endorsement is not possible before the announced closing date. In such cases, the NASA sponsoring office should be advised when a decision on endorsement can be expected.

(3) Successful and unsuccessful foreign entities will be contacted directly by the NASA sponsoring office. Copies of these letters will be sent to the foreign sponsor.. Should a foreign proposal or a U.S. proposal with foreign participation be selected, NASA's Office of External Relations will arrange with the foreign sponsor for the proposed participation on a no-exchange-of-funds basis, in which NASA and the non-U.S. sponsoring agency or funding institution will each bear the cost of discharging their respective responsibilities.

(4) Depending on the nature and extent of the proposed cooperation, these arrangements may entail:

(i) An exchange of letters between NASA and the foreign sponsor; or

(ii) A formal Agency-to-Agency Memorandum of Understanding (MOU).

M. Cancellation of NRA.. NASA reserves the right to make no awards under this NRA and to cancel this NRA. NASA assumes no liability for canceling the NRA or for anyone's failure to receive actual notice of cancellation.

NASA RESEARCH ANNOUNCEMENT (NRA) SCHEDULE

CELLULAR AND MACROMOLECULAR BIOTECHNOLOGY

All proposals submitted in response to this Announcement are due on the date and at the address given below by the close of business (4:30 PM EDT). NASA reserves the right to consider proposals received after this deadline if such action is judged to be in the interest of the U.S. Government. A complete schedule of the review of the proposals is given below:

NRA Release Date: August 4, 2000

Notice of Intent Due: September 6, 2000

Proposal Due: October 27, 2000

Submit Proposal to:

Dr. Steve Davison
c/o NASA Peer Review Services
Subject: NASA Research Proposal (NRA-00-HEDS-03)
500 E Street, SW, Suite 200
Washington, DC 20024
Telephone number for delivery services: (202) 479-9030

Final Selections: February, 2001

Funding commences: March, 2001

(dependent upon actual selection and procurement process)

FORMS PACKAGE FOR RESPONDING TO THIS NRA:

- **FORM A:** Solicited Proposal Application

- **FORM B:** Proposal Executive Summary

  The executive summary should succinctly convey, what it is the proposer wants to do, how the proposer plans to do it, why it is important, how it meets the requirements for microgravity relevance or supports HEDS research objectives. In addition, check the box that defines the proposal as Ground-based, Flight Definition or Interdisciplinary Project.

- **FORM C:** Budget For Entire Project Period Direct Costs Only

- **FORM D:** Summary Proposal Budget - 1 copy for each year)

- **FORM E:** Proposer Checklist of materials required for proposal submission